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[1]Research Center for Food Safety and Nutrition, Key Lab of Urban Agriculture (South), Bor S. Luh Food Safety Research Center, School of Agriculture and Biology, Shanghai Jiao Tong University, Shanghai, China
[2]Zhenjiang Institute of Agricultural Sciences in Hilly Area of Jiangsu Province, Jiangsu, China
[3]Department of Nutrition and Health, YIHS Institute of Biomedical Technology Co. Ltd., Jiangsu Vocational College of Medicine, Jiangsu, China

Phytochemical composition, antioxidant capacity and ACE-inhibitory activity of China-grown radish seeds

Bingjun Qian[1, 3†], Yueping Pan[2†], Zhan Cai[1], Pu Jing[1*]

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Summary

The seeds of nine China-grown radish cultivars were analyzed for their phytochemical composition, antioxidant properties and ACE-inhibitory activity. Radish seeds contained 36.87-43.06% (w/w) oils, whereas 64.55-69.26% of the fatty acids were monounsaturated and 20.33-25.11% were polyunsaturated. The levels of δ-tocopherol (552.24-670.31 µg/g seed oils) and lutein (4.82-8.95 µg/g seed oils) differed in cultivars. The nine cultivars varied in total phenolics, flavonoids, and free phenolic acids, but not in proanthocyanidins. Seed extracts of Hybrid #63, Tou Xin Hong, and Hybrid #72 showed stronger DPPH radical scavenging capacity, ORAC, and FRAP than others ($p<0.05$). The Yanzhi #2 extracts exhibited a strong ACE-inhibitory activity, which was positively correlated with vanillic acid contents ($r = 0.890$, $p = 0.001$). It provides evidence on developing value-added utilization of radish seeds or seed fractions such as oil and flour as nutraceuticals or functional food ingredients.

Keywords: Radish seeds; Phenolics; Flavonoids; Antioxidants; ACE-inhibitory activity

Introduction

Radish (*Raphanus sativus* L.) is a root vegetable crop that is native to Europe and Eastern Asia (Muminović et al., 2005). The consumption of Radish has increased during the last decade due in part to recognition of their nutritional values and antioxidant properties (Lugasi et al., 1998; Vitória et al., 2001). It reported that radish consumption could reduce the risk of lung and colorectal cancers (Martinez-Villaluenga et al., 2010), and minimize genotoxicity and cytotoxicity (Hassan et al., 2011).

The radish seeds have gained considerable attention of countries such as Brazil, Turkey and Poland, due to their high levels of unsaturated fatty acids (Uluata and Özdemir, 2012; Ávila and Sodré, 2012; Kaymak, 2015) and other antioxidant compounds, such as phenolic acids and flavonoids (Pająk et al., 2014). So, the extracts from the radish seeds or seed flour could be potent functional food ingredients. A recent study reported that total seed oil (43%, w/w) of Turkey-grown radish consists of 62% monounsaturated fatty acids (MUFAs) and 17% polyunsaturated fatty acids (PUFAs) and radish seed oil contains significantly high levels of tocopherols (Uluata and Özdemir, 2012). Compared to selected seeds including mung bean, broccoli, and sunflower seeds, radish seeds in Poland contain higher levels of total phenolics and flavonoids (Pająk et al., 2014). Additionally, radish seeds contain functional proteins with *in vitro* antifungal activity (Terras et al., 1992).

About 17.5 million people die each year from cardiovascular diseases (CVDs), an estimated 31% of all deaths worldwide (http://www.who.int/cardiovascular_diseases/en). Hypertension and chronic inflammation, both of which can be caused by oxidation *in vivo*, are regarded as risk factors of CVD. Many studies showed that the secondary metabolites from plants are the potential compounds for preventing CVD, possible attributable to their antioxidant and angiotensin-converting enzyme (ACE)-inhibitory activity (Vasanthi et al., 2012; Balasuriya and Rupasinghe, 2011). Loizzo et al. (2007) isolated six flavonoids with ACE-inhibitory activity from *Ailanthus excelsa* (Roxb), of which kaempferol-3-O-β-galactopyranoside possesses the highest activity with an IC_{50} value of 260 µmol/L. Ojeda et al. (2010) isolated and identified two ACE inhibiting anthocyanins, delphinidin-3-O-sambubioside and cyanidin-3-O-sambubioside, from the aqueous extract of *Hibiscus sabdariffa* with IC_{50} of 84.5 and 68.4 g/mL, respectively. Most of the researches have been targeted at bioactive compounds from natural resources. It is almost universally accepted that the phytochemicals are the new alternatives for replacing the drugs to avoid the adverse side effects initiated by long use of the ACE inhibitors, such as captopril and Lisinopril.

There are several radish cultivars grown in different regions of China. White round, Korean white, Japan white, and White pink are the radish cultivars with high consumption, and Tou Xin Hong, Xin Lin Mei, and Yanzhi #2 are the radish cultivars whose roots are rich in pigments we have investigated (Jing et al., 2012), and Hybrid #63 and Hybrid #72 are new hybrid radish cultivars which have been created by Prof. Pan. However, few studies have evaluated their phytochemical compositions and antioxidant activities in seeds. Therefore, the objective of this study was to investigate the levels of fatty acids, tocopherols, carotenoids, total phenolics, flavonoids, and proanthocyanidins in the seeds of these nine radish cultivars and to evaluate the antioxidant capacity and ACE-inhibitory properties of these radish seeds.

Materials and methods

Radish seeds and chemicals

The nine radish cultivar were cultivated with conventional plantation measure, and their seeds were harvested by Zhenjiang Institute of Agricultural Sciences in Hilly Area of Jiangsu Province and donated to this experiment. Standards of Supelco 37 Component FAME Mix, α, β, γ, δ-tocopherols, β-carotene, lutein, cryptoxanthin, and zeaxanthin, 2,4,6-tripyridyl-S-triazine(TPTZ),6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), Folin-Ciocalteu reagent, 2,2′-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), and 2,2-diphenyl-1-picryhydrazyl radical (DPPH), Angiotensin-Converting Enzyme (ACE , EC 3.4.15.1) from rabbit lung, Hippuryl-His-Leu (HHL), and sinapic, vanillic, syringic, and ferulic

* Corresponding author
† These authors made equivalent contributions to the manuscript.

acid were purchased from Sigma-Aldrich (St. Louis, MO, USA). Gallic acid, (±)-catechin hydrate, cyanidin chloride were purchased from Aladdin (Shanghai, China).

All other chemicals and solvents were of the highest commercial grade and used without further purification. All other chemicals were purchased from Sinopharm Chemical Reagent (Shanghai, China).

Extraction of radish seeds

The oils and antioxidant extracts were prepared according to the previous description by JING et al. (2012). Radish seeds were ground into powder with a standard household coffee grinder until they could pass through a 40-mesh screen. Five grams of radish seed powder were extracted in 80 mL hexane for 6 h in a Soxhlet device. The hexane in the oil was evaporated using a rotary evaporator (Rotavapor RE-52, Yarong Inc., Shanghai, China) at reduced pressure. The oils were collected and stored at -20 ºC until analysis. Each sample was analyzed in three technical repeats. The radish seed flour (RSF) after oil extraction was then dried overnight under hood and weighed, where the weight loss was equal to the mass of extracted oils.

One gram of the flour was shaking in water bath with 10 mL of 50% acetone at room temperature for 2 h. Then, the extracts were centrifuged at 3000 g for 8 min. The prepared extracts were kept in the dark at 4 ºC for further analysis of antioxidants, such as phenolic acid, flavonoids, and proanthocyanidins. Each sample was analyzed in three technical repeats.

Analysis of FA composition by gas chromatography (GC)

Fatty acid methyl esters (FAME) were prepared by the KOH–methanol method (JING et al., 2012) prior to analysis of GC. Briefly, dissolving 20 mg oil in 2 mL iso-octane followed by another addition of 0.2 mL of 1 mol/L KOH–methanol. After a reaction at room temperature for 5 min, 2 mL of iso-octane and approximately 3 mL water were successively added. Then, removing the supernatant and washing the residues with 3 mL water twice. The upper iso-octane layer was collected and subjected to fatty acid analysis using gas chromatography (Shimadzu GC-2010), which was equipped with an Omegawax column (30 m × 0.25 mm with a 0.25 μm film thickness) from Supelco (Bellefonte, PA, USA) and a flame ionization detector (FID). The carrier gas was Helium, and the flow rate was set as 1.0 mL/min. Oven temperature-rising program was set as follows: initially 50 ºC for 2 min, increasing to 220 ºC at 30 ºC/min and holding at 220 ºC for 20 min, followed by heating from 220 to 260 °C at 10 °C/min and holding at 260 °C for 10 min. Each sample was analyzed in three technical repeats. The various fatty acids were identified according to the standards mixture (Supelco 37 Component FAME Mix, Supelco, PA, USA). The concentration of each fatty acid was expressed as a percentage of total area of all fatty acid peaks.

Determination of tocopherol contents of oil from seed by Ultra Performance Liquid Chromatography (UPLC)

The separation samples were prepared by dissolving about 200 mg oil in 10 mL methanol/tetrahydrofuran (THF) (1:1, v/v) prior to analysis of tocopherol contents in seed oil. The samples were separated on a Waters ACQUITY UPLC-C_{18} column (100 mm × 2.1 mm, 1.7 μm particle size) using an ACQUITY Ultra Performance Liquid Chromatography (UPLC) system with a TUV detector (Waters, Milford, USA) according to a previous method (JING et al., 2012). Elution was performed at a flow rate of 0.3 mL/min with a binary gradient (a mobile phase of water was used as solvent A, and acetonitrile was used as solvent B) of solvent B in A going from 80% to 99% over 5 min followed by 99% for 5 min. Tocopherols were monitored at 294 nm and identified according to the UPLC retention time with those of tocopherol standards. The standard curves were constructed for quantification. Each sample was analyzed in three technical repeats.

Analysis of Carotenoid compositions by Ultra Performance Liquid Chromatography (UPLC)

The separation samples prepared was also loaded on C-30 YMC carotenoid column (YMC, Wilmington, NC; 150 × 4.6 mm, 5 μm particle size) using the ACQUITY Ultra Performance Liquid Chromatography (UPLC) system equipped with a TUV detector according to a previous method (JING et al., 2012). The mobile phase was methanol/methyl tertiary butyl ether (MTBE)/H_2O (81:15:4, v/v/v) as solvent A and MTBE/methanol (91:9, v/v) as solvent B. Elution was performed at a flow rate of 1 mL/min with a binary gradient of solvent B in A going from 0% to 50% over 30 min, 100% for next 10 min, and 0% for next 5 min. Carotenoids were monitored at 450 nm and identified by comparing the UPLC retention time with standard (compounds containing β-carotene, lutein, cryptoxanthin, and zeaxanthin), and then quantified based on standard curves. Each sample was analyzed with three technical repeats.

Determination of total phenolic contents (TPC) of radish seed flour extract

Total phenolics of radish seed flour extract were measured using a modified Folin-Ciocalteu method (WATERHOUSE, 2001). Briefly, 50 μL of radish seed flour extract samples, gallic acid dilutions (standards), or water blank was added into each tube, respectively, which was filled with 3 mL of water and 250 μL of Folin-Ciocalteu reagent in advance. The sample were mixed well and placed at ambient temperature for 10 min. Then, 750 μL of 20% (w/v) Na_2CO_3 solution was added into each test tube and mixed well prior to reaction at ambient temperature for 2 h. Absorbance was read at 765 nm using a L5S UV/Vis spectrophotometer (Shanghai Analytical Instrument, China). Each test was performed in triplicate. Total phenolics were calculated as gallic acid equivalents (GAE) per gram of radish seeds based on a gallic acid standard curve.

Analysis of Phenolic acid composition by High Performance Liquid Chromatography (HPLC)

Soluble free phenolic acid compositions in each radish seed were analyzed with a previously reported procedure (JING et al., 2012). For quantitative analysis of phenolic acid composition in antioxidant extracts of radish seeds, the extracts were loaded on a Zorbax Eclipse XDB-C_{18} column (250 mm × 4.6 mm, 5 μm, Agilent Technologies, Palo Alto, CA, USA) using Agilent 1260 infinity HPLC system. Phenolic acids were separated at a flow rate of 1 mL/min with a binary gradient (mobile phase of formic acid/H_2O (0.1:99.9, v/v) was used as solvent A and mobile phase of formic acid/acetonitrile (0.1:99.9, v/v) was used as solvent B) with going from 0% to 7% over 5 min; from 7% to 25% B over 40 min; from 25% to 45% over 10 min. Phenolic acids were identified by comparing the retention time and spectrum of peaks in the samples to that of the standards under the same HPLC conditions. Each sample was analyzed in three technical repeats. Quantification of each phenolic acid was determined using external standards and total area under each peak.

Determination of total flavonoid contents (TFC) of radish seed flour extracts

The TFC of radish seed flour extracts were determined according to a description by JING et al. (2012). Thirty microliters of sample were added into a well of a 96-well plate and mixed with 180 μL of water followed by 10 μL of a 5% $NaNO_2$ solution was added and allowed

to stand for 6 min. Subsequently, 20 µL of a 10% $AlCl_3$ solution was added and allowed to stand for 6 min. Finally, 60 µL of 4% (w/v) NaOH solution were added into the mixture to stop the reaction for 15 min. Absorbance was measured at 510 nm using a microplate reader (Infinite F200 PRO; Tecan, Switzerland). Each test was performed in triplicate, and total flavonoids were calculated as catechin equivalents (CAE) of radish seed based on a catechin standard curve.

Determination of total proanthocyanidin content (TPCC) of radish seed flour extracts

The TPCC of radish seed flour extracts were determined using the HCl/n-butanol assay (Porter et al., 1985). Briefly, 0.5 mL of seed flour extract was added into test tubes containing 6 mL of a 95% solution of n-butanol/HCl (95:5, v/v) and mixed well. Subsequently, 0.2 mL of a solution of 2% (w/v) $NH_4Fe(SO_4)_2$ in 2 mol/L HCl was added into the mixture and incubated for 40 min at 90 ºC. The absorbance was measured at 550 nm in L5S UV/Vis spectrophotometer. TPCC was expressed as mg of cyanidin equivalents (CyE) per gram of radish seeds.

Evaluation of antioxidant capacity by chemical assays

DPPH radical scavenging capacity

Briefly, 100 µL of 0.2 mmol/L DPPH solution was mixed with 100 µL of radish seed flour 50% acetone extracts at different concentrations to initiate the reactions in each well of a 96-well plate. Absorbance at 515 nm was determined after 40 min of reaction in a microplate reader (Infinite F200 PRO; Tecan, Switzerland). The blank contained only 200 µL of solvent, and the control consisted of 100 µL of solvent and 100 µL of 0.2 mmol/L DPPH. The DPPH radical-scavenging activity in the extracts was expressed as micromoles of Trolox equivalents per gram of seed.

Oxygen Radical Absorbance Capacity (ORAC) assay

Determination of ORAC of the studied compounds was performed according to the previous description (Jing et al., 2014). Same as samples tested, Trolox standards were dissolved in 50% acetone. Other reagents were prepared in 75 mmol/L phosphate buffer (pH 7.4). Briefly, 30 µL of 20 µmol/L extracts (or 50% acetone for blank control) were mixed 225 µL fluorescein (81.63 nmol/L) in each well of a 96-well plate. Subsequently, the plate was covered and incubated at 37 °C for 20 min. To start reaction, 25 µL of 0.36 M 2,2′-azobis(2-amidinopropane) hydrochloride (AAPH) were added and mixed well with above mixture in each well. The fluorescence was recorded with 5 min internal for 2 h (ex/em: 485/538 nm) at 37 °C in a microplate reader (Infinite F200 PRO; Tecan, Switzerland). A concentration-area under the curve (AUC) stand curve of Trolox was constructed under the same experimental conditions and used for calculation of equivalents. Results are expressed as micromoles of Trolox equivalents per gram of radish seed. Reactions were conducted in triplicate.

The ferric reducing ability of plasma (FRAP) assay

The FRAP assay was determined based on the reduction of Fe^{3+}-TPTZ to a blue coloured Fe^{2+}-TPTZ (Benzie and Strain, 1996) with some modification. Three hundred millimoles per liter of acetate buffer (pH 3.6), 10 mmol/L TPTZ and 20 mmol/L $FeCl_3 \cdot 6H_2O$ were mixed together in a ratio of 10:1:1 (v/v/v) to produce the FRAP reagent. Then, 3 mL of FRAP reagent was added into the test tube containing 100 µL of sample or standards and 300 µL of water and mixed well by vortexing followed by an incubation at 37 °C for 30 min. Absorbance at 593 nm was measured using L5 UV spectrometer. Trolox was used as standard for comparison and adequate dilution of sample was performed. Results were reported as Trolox equivalents (TE) per gram of radish seed. Reactions were conducted in triplicate.

Determination of Angiotensin I converting enzyme (ACE) inhibitory activity

The ACE inhibitory activity was measured according to a description of Kurita et al. (2010). Twenty five microliters of prepared antioxidant extracts were pre-incubated with 50 µL of substrates (4.7 mmol/L hippuryl-His-Leu in 400 mmol/L potassium phosphate buffer (pH 8.3) containing 600 mmol/L NaCl) at 37 °C. Then 50 µL of 0.025 U/mL ACE solution were added followed by an incubation at 37 °C for 40 min. Aliquots (25 µL) of the reaction mixture were added into each well containing 150 µL of 0.3 mol/L NaOH and 10 µL of 2% *o*-phthalaldehyde in methanol of a 96-well microplate. Finally, the reaction was terminated by adding 20 µL of 3 mol/L HCl and kept at ambient temperature for 10 min. The fluorescence generated was recorded at excitation and emission wavelengths of 355 and 460 nm, respectively, in a microplate reader (Infinite F200 PRO; Tecan, Switzerland). For the control and blank, water was added instead of samples and ACE, respectively. The inhibition rate was calculated as the following:

$$\text{ACE inhibitory activity } \% = (1 - \frac{f_s - f_b}{f_c - f_b}) \times 100$$

Where f_s is the fluorescence intensity of the test sample in the presence of the reaction mixture, f_b is the fluorescence intensity of test sample in the absence of ACE, and f_c is the fluorescence intensity of buffer in the absence of the test sample. Each sample was analyzed in three technical repeats.

Statistical analysis

All results are expressed as the mean±SD. Univariate ANOVA among means of chemical contents, antioxidant activities, or ACE-inhibitory activities in nine radish seeds were performed by least significant difference (LSD) test in General Linear Model at the level of 0.05. Correlation among means was determined using a two-tailed Pearson correlation test. Statistics was analyzed using SPSS (version 14.0, SPSS Inc., Chicago, IL, USA).

Results and discussion

Total phenolics, flavonoids, and proanthocyanidins in radish seeds

The total phenolics, flavonoids, and proanthocyanidins levels in radish cultivars are shown in Tab. 1. Total phenolic levels varied from 9.15 mg (in Hybrid #63) to 14.54 mg (in White pink) gallic acid equivalents (GAE)/g dry mass (DM). Among the cultivars, White pink seeds had the highest total phenolic levels (14.54 mg GAE/g DM; $p<0.05$). In this study, the total phenolic levels obtained were higher than those previously reported in radish seeds of 6.7 or 6.1 mg GAE/g DM (Pajak et al., 2014; Aguilera et al., 2015) and in pomegranate seeds of 1.29-2.17 mg GAE/g DM (Jing et al., 2012). The differences in results could be attributed to the species, cultivars, growing conditions, genotypes, and extraction methods. The results revealed that the tested radish seeds had high levels of total phenolic compounds. The total flavonoids in radish seeds varied from 0.51-1.36 mg catechin equivalents (CAE)/g DM (Tab. 1). Tou Xin Hong seeds contained the highest level of total flavonoids (1.36 mg CAE/g DM) followed by White pink and Yanzhi #2 (~1.28 mg CAE/g DM; $p>0.05$). It has been reported that radish leaves contain >200 mg CAE/g DM flavonoids (Kim et al., 2014), which is considerably higher than the level present in radish seeds. Total proanthocyanidin levels

Tab. 1: Total phenolics, flavonoids, and proanthocyanidins in radish seeds

Cultivars	Phenolics (mg GAE/g)	Flavonoids (mg CE/g)	Proantho-cyanidins (mg CyE/g)
White round	11.60 ± 0.25^{ab}	0.77 ± 0.10^{abcd}	1.15 ± 0.10^{a}
Korean white	9.34 ± 0.59^{ab}	0.68 ± 0.06^{abcd}	1.12 ± 0.35^{a}
Japan white	9.84 ± 0.53^{ab}	0.86 ± 0.07^{bcd}	0.82 ± 0.17^{a}
Hybrid #63	9.15 ± 0.94^{a}	0.51 ± 0.07^{a}	0.90 ± 0.01^{a}
Hybrid #72	9.93 ± 0.25^{ab}	0.90 ± 0.17^{cd}	0.79 ± 0.21^{a}
White pink	14.54 ± 2.76^{c}	1.28 ± 0.14^{e}	0.87 ± 0.14^{a}
Tou Xin Hong	10.79 ± 0.61^{ab}	1.36 ± 0.10^{e}	1.10 ± 0.23^{a}
Xin Lin Mei	10.51 ± 0.23^{ab}	0.61 ± 0.06^{abc}	0.74 ± 0.24^{a}
Yanzhi #2	12.21 ± 0.81^{bc}	1.28 ± 0.10^{e}	1.11 ± 0.26^{a}

Values are expressed as mean ± SD (n=3). Different letters within a column represent significant differences ($p<0.05$). GAE: gallic acid equivalents; CE: catechin equivalents; CyE: cyanidin equivalents

ranged from 0.74-1.15 mg cyanidin equivalents (CyE)/g DM (Tab. 1). Similar levels have been reported in pomegranate seeds of 0.68-1.82 mg CyE/g DM (Jing et al., 2012).

Total oil levels and fatty acid composition

The fatty acid compositions of radish seed oils are shown in Tab. 2. Total oil levels (36.87-43.06 g/100 g seeds) in dry China-grown radish seeds were higher than or comparable to those (22.6-37.9 g/100 g seeds or 42.64 g/100 g seeds) in Turkey-grown radish seeds reported by Uluata and Özdemir (2012) or Kaymak (2015), repectively. The China-grown radish seed oils consisted of 89% (w/w) unsaturated fatty acids, composing of 64.55-69.26% MUFA and 20.33-25.11% PUFA in Tab. 2. All were higher than those grown in Turkey (Uluata and Özdemir, 2012).

The predominant MUFAs were erucic (C22:1, 34.53-40.32%) and oleic (C18:1, 16.20-21.30%) acids that were slightly higher than or comparable to the previous reports by Uluata and Özdemir (2012) or Kaymak (2015), who found 22.1-36.4%, or 40.83% erucic and 12.6-23.9%, or 19.08% oleic acids in radish seed oils, respectively. The levels of linoleic (C18:2, 10.14-14.28%) and linolenic (C18:3, 8.40-11.17%) acids as major PUFAs were comparable to those as 10.09% and 7.02% for each in previous literatures (Uluata and Özdemir, 2012; Kaymak, 2015).

Tocopherols and carotenoids

Tocopherols and carotenoids were detected in seeds oils of tested radish cultivars in Tab. 3. The δ-tocopherol varied from 552.24-670.31 μg/g seed oils, whereas α-, γ-, or β-tocopherols were not detected. The level of δ-tocopherol in China-grown radish seeds was greater than those in Turkey-grown radish seeds reported as 545.67 μg/g seed oils by Uluata and Özdemir (2012), who also found α-, γ-, and β-tocopherols as 12.41-28.66 μg/g seed oils in Turkey radish seeds. Tou Xin Hong (670.31 μg/g seed oils) and White pink (656.60 μg/g seed oils) contained greater amount of δ-tocopherol than other seven cultivars in this study ($p<0.05$).

Among the carotenoids, only lutein was detected with levels ranging from 4.82-8.95 μg/g seed oils. Hybrid #63 seeds among all cultivars contained the greatest amount of lutein ($p<0.05$), comparable to one in soybeans of 1.6-14.8 μg/g seed oils (Kanamaru et al., 2006).

Free phenolic acid composition

The sinapic, vanillic, syringic, and ferulic acid were found in the seed flours of nine tested cultivars in Tab. 4. A study reported that gallic,

Tab. 2: Fatty acid composition and total oil content of radish seeds

Cultivars	Percentage of fatty acids (%)																Total oil (g/100g seeds)
	16:0	16:1	18:0	18:1	18:2	18:3	20:0	20:1	20:2	22:0	22:1	22:6	24:0	SFA	MUFA	PUFA	
White round	4.82 0.12	0.18 0.01	1.71 0.12	19.32 0.14	14.08 0.35	9.46 0.14	1.15 0.02	9.76 0.11	0.58 0.05	0.93 0.02	35.44 0.52	0.67 0.10	1.90 0.13	10.51^{a} 0.45	64.70^{a} 0.17	24.79^{a} 0.20	38.65^{cd} 0.67
Korean white	4.67 0.11	0.17 0.02	1.81 0.23	20.70 0.23	10.68 0.22	8.40 0.11	1.37 0.05	11.44 0.31	0.48 0.02	1.15 0.10	36.95 0.47	0.77 0.02	1.40 0.14	10.40^{a} 0.31	69.26^{b} 0.10	20.33^{b} 0.02	40.90^{abc} 0.90
Japan white	4.50 0.15	0.15 0.02	1.58 0.12	16.20 0.08	14.28 0.25	9.24 0.20	1.15 0.22	7.86 0.20	0.64 0.03	1.27 0.23	40.32 0.53	0.95 0.01	1.86 0.02	10.37^{a} 0.22	64.55^{a} 0.03	25.11^{a} 0.02	43.06^{a} 0.30
Hybrid #63	4.62 0.05	0.15 0.04	1.97 0.03	21.77 0.31	12.02 0.31	10.40 0.22	1.36 0.13	9.72 0.31	0.47 0.01	1.18 0.15	34.53 0.22	0.60 0.04	1.21 0.21	10.34^{a} 0.41	66.17^{c} 0.04	23.49^{a} 0.06	37.28^{d} 0.26
Hybrid #72	4.70 0.02	0.16 0.01	1.71 0.08	18.40 0.14	13.76 0.24	8.57 0.32	1.21 0.05	8.55 0.25	0.56 0.12	1.22 0.20	38.50 0.30	0.90 0.11	1.74 0.23	10.58^{a} 0.32	65.61^{ac} 0.15	23.79^{a} 0.07	39.75^{bc} 0.70
White pink	5.11 0.16	0.27 0.13	1.69 0.07	19.36 0.23	12.93 0.15	8.41 0.15	1.25 0.07	9.76 0.32	0.56 0.01	1.06 0.02	36.61 0.51	0.77 0.03	1.62 0.02	10.73^{a} 0.14	66.00^{c} 0.86	22.67^{ab} 0.27	42.82^{a} 0.28
Tou Xin Hong	4.88 0.05	0.18 0.11	1.59 0.05	19.98 0.23	13.67 0.41	8.60 055	1.03 0.23	8.93 0.41	0.52 0.03	0.91 0.12	36.94 0.45	0.75 0.12	2.04 0.14	10.45^{a} 0.22	66.03^{c} 0.09	23.54^{a} 0.05	36.87^{d} 0.96
Xin Lin Mei	4.70 0.08	0.19 0.08	1.91 0.13	21.30 0.13	10.14 0.52	11.17 0.62	1.36 0.14	9.80 0.35	0.44 0.02	1.17 0.23	35.79 0.38	0.67 0.23	1.36 0.05	10.50^{a} 0.12	67.08^{d} 0.12	22.42^{ab} 0.07	40.24^{bc} 0.49
Yanzhi #2	4.72 0.06	0.17 0.05	1.67 0.22	19.55 0.24	13.75 0.63	9.32 0.45	1.14 0.06	9.84 0.51	0.57 0.06	0.93 0.05	35.81 0.39	0.70 0.21	1.84 0.21	10.30^{a} 0.43	65.37^{a} 0.14	24.34^{a} 0.06	41.19^{ab} 0.45

Oils were extracted for 6 h from 40-mesh ground seeds by Soxhlet extraction. Data expressed as mean and standard deviation (n=3). SFAs: saturated fatty acids. MUFAs and PUFAs represent monounsaturated fatty acids and polyunsaturated fatty acids, respectively. Different letters within a column represent significant differences ($p<0.05$).

Tab. 3: Tocopherol and lutein contents of radish seed oils[1]

Cultivars	δ-Tocopherol (μg/g oils)	Lutein (μg/g oils)
White round	562.24 ± 61.27^{a}	7.90 ± 0.34^{c}
Korean white	579.92 ± 15.90^{a}	6.20 ± 0.08^{b}
Japan white	585.15 ± 47.90^{a}	8.55 ± 0.35^{d}
Hybrid #63	591.55 ± 46.92^{a}	8.95 ± 0.31^{e}
Hybrid #72	610.16 ± 32.94^{ab}	8.29 ± 0.10^{cd}
White pink	656.60 ± 35.20^{b}	5.98 ± 0.08^{b}
Tou Xin Hong	670.31 ± 25.78^{b}	8.31 ± 0.13^{d}
Xin Lin Mei	578.25 ± 14.88^{a}	7.55 ± 0.12^{c}
Yanzhi #2	552.24 ± 25.97^{a}	4.82 ± 0.06^{a}

[1]Oils were extracted for 6 h from powder of radish seeds by the Soxhlet method. δ-tocopherol was quantified by HPLC. Results are expressed as μg/g oils. Values are expressed as mean±standard deviation (n = 3). Different letters within a column represent significant differences ($p<0.05$).

protocatechuic, caffeic, *p*-coumaric, and ferulic acids were detected as free phenolic acids in radish seeds (Pajak et al., 2014). Ferulic acid was the predominant free phenolic acid (33.40-47.59 μg/g seeds) in tested radish seeds, much greater than those in radish seeds *var.* Flamboyant 2 (ferulic acid, 21 μg/g seeds) (Pajak et al., 2014). Yanzhi #2 contained the highest levels of vanillic acid (18.77 μg/g seeds, $p<0.05$) among all tested seeds.

Antioxidant capacity

More than one radical system was applied to investigate the radical scavenging capacities of radish seed extracts and results are shown in Fig. 1. The 50% acetone extracts of radish seed flour (RSF) were used to quench DPPH radical in the testing system. The DPPH radical scavenging capacity of RSF from 14.84-26.35 μmol TE/g seeds in Fig. 1A, was much greater than those in previous studies reported as 12 μmol TE/g (Pajak et al., 2014) and 1.4 μmol TE/g (Aguilera et al., 2015). Among all tested cultivars, the Hybrid #63 showed the highest levels ($p<0.05$).

The oxygen radical absorbance capacity of RSF extracts was determined using the ORAC assay. Fig. 1B shows the ORAC values of RSF from nine tested cultivars. Tou Xin Hong had the high ORAC value (36.32 μmol TE/g seeds), followed by White round, White pink, Hybrid #72, and Yanzhi #2. Hybrid #63 exhibited the lowest value (16.67 μmol TE/g seeds) among all cultivars ($p<0.05$).

The reducing power of RSF extracts were evaluated using the FRAP test. Fig. 1C shows that Hybrid #72 exhibited the higher FRAP value as 1.04 μmol TE/g seeds than other cultivars that showed no differences. Generally, the FRAP values of nine radish cultivar seeds were lower than those reported as 30.6 μmol TE/g DW seeds (Aguilera et al., 2015).

Positive correlations were found between total phenolics and flavonoids ($r = 0.679$, $p = 0.044$), proanthocyanidins and phenolic acids ($r = 0.754$, $p = 0.019$), flavonoids and ORAC ($r = 0.668$, $p = 0.049$). Wolfe et al. (2008) also described that total phenolics from common fruits were significantly correlated to ORAC values ($r = 0.761$, $p<0.05$). In this study, we only evaluated the content of free phenolic acids, while majority of them may be present in radish seeds as a bound forms (i.e, esters and glycosides), which might be a main reason for lack of correlation between free phenolic acids and antioxidant activities. It is easy to understand that as the phenolics and flavonoids can quench or prevent the formation of reactive oxygen species and reactive nitrogen species. Therefore, plant phenolics and flavonoids are critical in preventing various diseases associated with oxidative stress, such as cardiovascular diseases (Manach et al., 2005). Plant phenolics could reduce oxygen radicals and increase the bioavailability of NO to promote vasodilation (Schewe et al., 2008). Additionally, plant flavonoids could promote RNA expression of endothelial nitric oxide synthase to increase the level of NO attributing to decreasing blood pressure (Vasanthi et al., 2012). So, it suggested that the extracts of RSF exhibited a potential role in promoting cardiovascular health.

ACE-inhibitory activity

Angiotensin I converting enzyme (ACE) catalyzes the conversion of angiotensin I into angiotensin II, a strong vasoconstrictor that increases blood pressure (Skeggs et al., 1956). Many compounds were reported to exert both of antioxidant activity and ACE-inhibitory activity (Chopra et al., 1992; Pihlanto et al., 2008). Therefore, the nine China-grown radish seeds were evaluated for their inhibitory effect on ACE, based on 1-mL extracts obtained from 2 mg of seed flour. Yanzhi #2 inhibited 100% of ACE activity, followed by Hybrid #63 (40.16%; Fig. 2). Additionally, Yanzhi #2 still exhibited 34.72% of ACE inhibitory activity following a five-fold dilution, which remained higher than the ACE-inhibitory activity of other extracts with the exception of Hybrid #63 ($p<0.01$).

Positive correlations were found between vanillic acid and ACE-

Tab. 4: Free phenolic acids in radish seeds[1]

Cultivars	Sinapic	Vanillic	Syringic	Ferulic	Total
White round	3.78±0.14^{a}	ND	4.87 ± 0.45^{ab}	47.59 ± 1.01^{e}	56.24 ± 1.57^{e}
Korean white	3.62±0.45^{a}	1.99 ± 0.28^{b}	6.94 ± 0.70^{a}	47.75 ± 1.86^{e}	61.35 ± 2.98^{e}
Japan white	3.23±0.13^{a}	1.32 ± 0.08^{b}	1.90 ± 0.26^{ab}	34.47 ± 1.02^{c}	40.92 ± 1.49^{c}
Hybrid #63	3.00±1.11^{a}	ND	ND	20.18 ± 1.73^{a}	23.18 ± 1.84^{a}
Hybrid #72	3.22±0.15^{a}	0.46 ± 0.01^{b}	3.03 ± 0.25^{ab}	27.91 ± 1.65^{b}	34.62 ± 2.06^{b}
White pink	2.82±0.30^{a}	1.29 ± 0.05^{b}	1.54 ± 0.13^{b}	33.40 ± 1.42^{c}	39.05 ± 1.90^{b}
Tou Xin Hong	3.65±0.12^{a}	2.02 ± 1.43^{b}	3.73 ± 0.12^{ab}	40.43 ± 2.43^{d}	49.83 ± 2.81^{d}
Xin Lin Mei	3.52±0.22^{a}	1.25 ± 1.25^{b}	4.29 ± 0.56^{ab}	35.70 ± 2.31^{cd}	41.24 ± 3.21^{c}
Yanzhi #2	ND	18.77 ± 1.65^{a}	4.55 ± 0.43^{ab}	46.77 ± 3.20^{e}	70.09 ± 5.28^{f}

[1]Sinapic, vanillic, syringic, and ferulic stand for sinapic, vanillic, syringic, and ferulic acids. Results expressed as micrograms of individual standard per gram of radish seeds. Data expressed as mean±standard deviation (n = 3). Different letters within a column represent significant differences ($p<0.05$); ND, not detected.

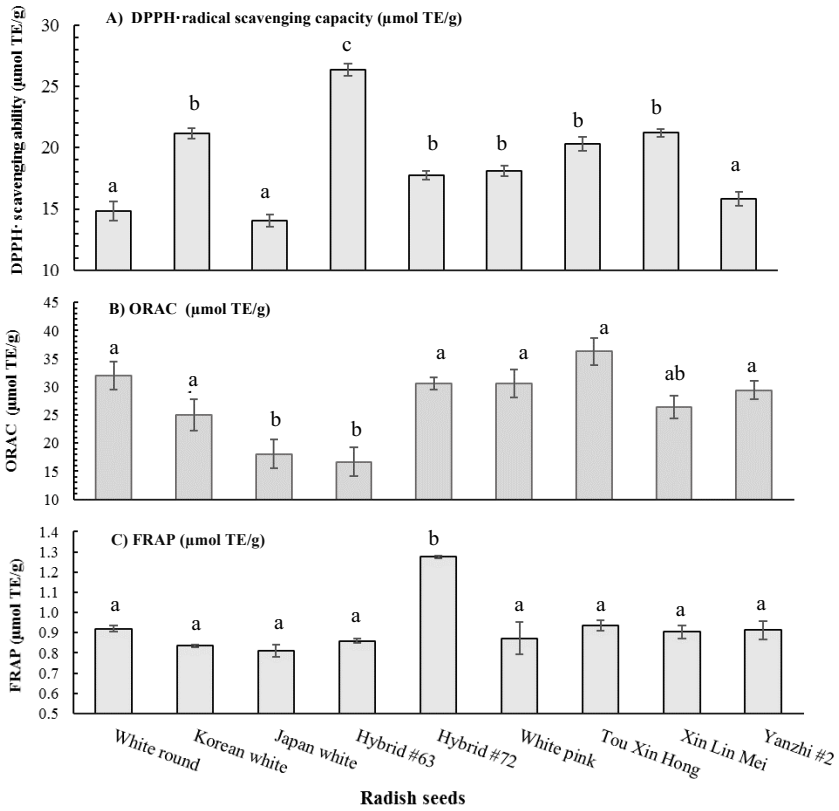

Fig. 1: Antioxidant activities of radish seeds: A) DPPH· radical scavenging capacity values; B) ORAC values; C) FRAP. Results expressed as μmol Trolox equivalents (TE)/g RSF. Tests were conducted in triplicate. Vertical lines represent standard deviations. Columns marked with different letters are significantly different ($p<0.05$).

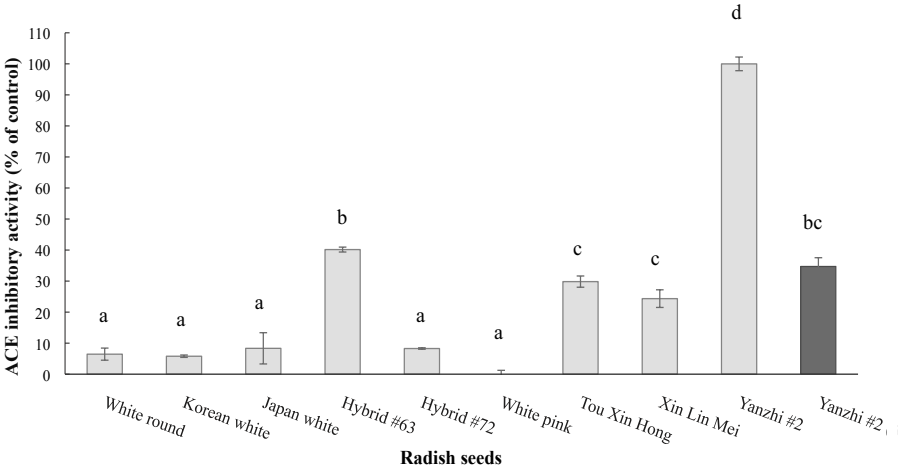

Fig. 2: ACE inhibitory activities of antioxidant extracts of radish seeds. ACE inhibitory activity was measured from 1-ml extracts obtained from 2 mg of seed flour except for Yanzhi #2 (5D), which had a five-fold dilution. Tests were conducted in triplicate. Vertical lines represent standard deviations. Columns marked with different letters are significantly different ($p<0.05$).

inhibitory activity ($r = 0.890$, $p = 0.001$). There were no correlations between antioxidant activity and ACE-inhibitory activity, or with vanillic acid. Another report also described the similar results, in which intake of flavonoid-rich apple peel extract did not cause significant differences in serum and lung ACE activity at week eight but reduced blood pressure after 5 weeks of treatment in spontaneously hypertensive rats (SHR) possibly through endogenous antioxidant pathways (Balasuriya et al., 2015). The IC_{50} of vanillic acid for ACE-inhibitory activity was about 8 mmol/L, which linear correlated to the molecular docking score between phenolic compounds and testicular ACE (Al Shukor et al., 2013). The ACE-inhibitory activity of vanillic acid was proposed to be charge-charge interactions with the zinc ion in the active site of ACE (Al Shukor et al., 2013). Therefore, vanillic acid might be responsible for the ACE-inhibitory activity of radish seeds but not for their antioxidant activities like phenolics and flavonoids (Schewe et al., 2008; Vasanthi et al., 2012). Certainly, as mentioned in the introduction, some flavonoids and anthocyanins also had ACE inhibitoring activity (Loizzo et al., 2007; Ojeda et al., 2010). On the other hand, compounds with sulfhydryl groups are effective as free radical scavengers and as ACE

inhibitors (Terras et al., 1992). So, it could not be overlooked that the ACE inhibitory activity of the extracts in this study was also associated with some other phytochemicals.

Conclusions

The results of this study confirmed that radish seeds are good sources of unsaturated fatty acids, tocopherols, lutein, and antioxidants. Radish cultivars may differ in seed composition and health properties; seed composition might be dependent on the geographical location. This study provides important information for developing value-added utilization of radish seeds or seed fractions such as oil and flour as nutraceuticals or functional food ingredients.

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Conflict of interest
Authors declare no conflict of interest.

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Address of the corresponding author:
E-mail: pjing@sjtu.edu.cn